

Supporting Information

Visible light initiated thiol-acrylate photopolymerization of heparin-based hydrogels

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Additional Information on Micropatterning

Preparation of release layer on the glass slide.

In order to enable controlled retrieval of micropatterned heparin based hydrogels from their substrate, one of the glass slides is treated to provide an initially-adhesive surface that can be subsequently “switched” to release the gel in a stimuli-response manner.¹ Specifically, a layer by layer (LbL) technique was used to deposit a (PLL-HA)₂ multilayer followed by an adhesive capping layer. First, the glass slides were cleaned by ultrasonication in isopropanol and deionized water for 10 minutes in each. The glass slides were dried with filtered nitrogen and exposed to an oxygen plasma (YES-R3, San Jose, CA, USA) for 5 minutes. The polyelectrolytes were adsorbed on the glass using the usual layer-by-layer technique immersing the substrate alternately in the polycation and the polyanion solutions with rinsing in between. Specifically, the glass slide is first placed in a PLL (0.5 mg/ml) solution for 5 minutes, followed by rinsing with PBS, then it is placed in a HA (0.5 mg/ml) solution, followed by rinsing with PBS,. This procedure was repeated one more time to create a (PLL-HA)₂ multilayer. As the last adsorption step, acryl-functionalized chitosan (GMA-Chi, 30% acrylation) (5 kDa) was adsorbed by immersing in a GMA-Chi (30% acrylation, 2 mg/ml) solution for 30 minutes, followed by rinsing with PBS. GMA-Chi was synthesized as previously reported.² Modified glass slides were placed in a desiccator until further use. The glass substrates bearing this multilayer are denoted as (PLL-HA)₂-(GMA-Chi).

¹ Gwon, K.; Kim, M.; Tae, G. *Integr. Biol.* **2014**, *6*, 596-602.

² Kim, J. Y.; Choi, W. I.; Kim, Y. H.; Tae, G.; Lee, S. Y.; Kim, K.; Kwon, I. C. *J. Controlled Release* **2010**, *147*, 109-117.

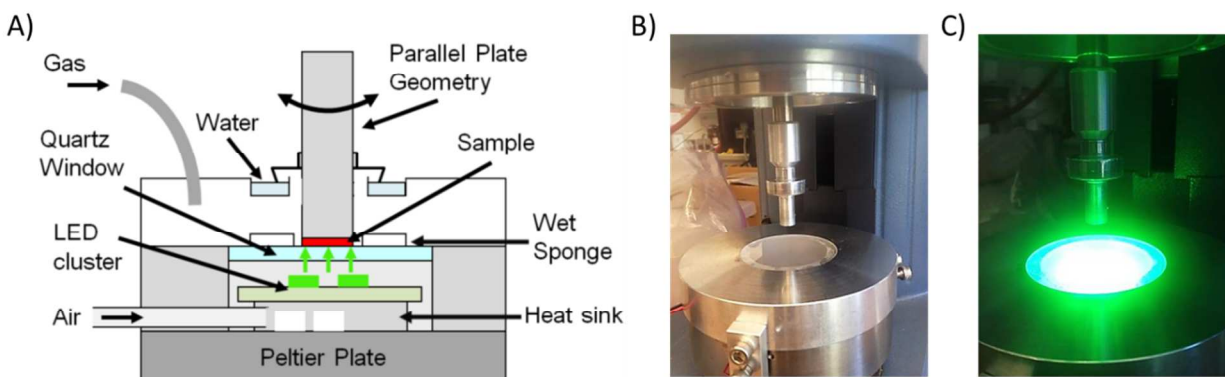


Figure S1. A) Diagram of photo-rheology apparatus. The green LED cluster is mounted on a ventilated heat sink that removes heat generated from the LED to prevent the sample from heating when the LED is on. The LED assembly is mounted beneath the transparent bottom plate of the rheometer, used in a parallel plate geometry. The hydrogel (“Sample,” shown in red) is prevented from drying by surrounding the sample with a wet sponge during the experiment. Photographs of the rheometer (without sample and environmental control) when the LEDs are turned off (B) and turned on (C).

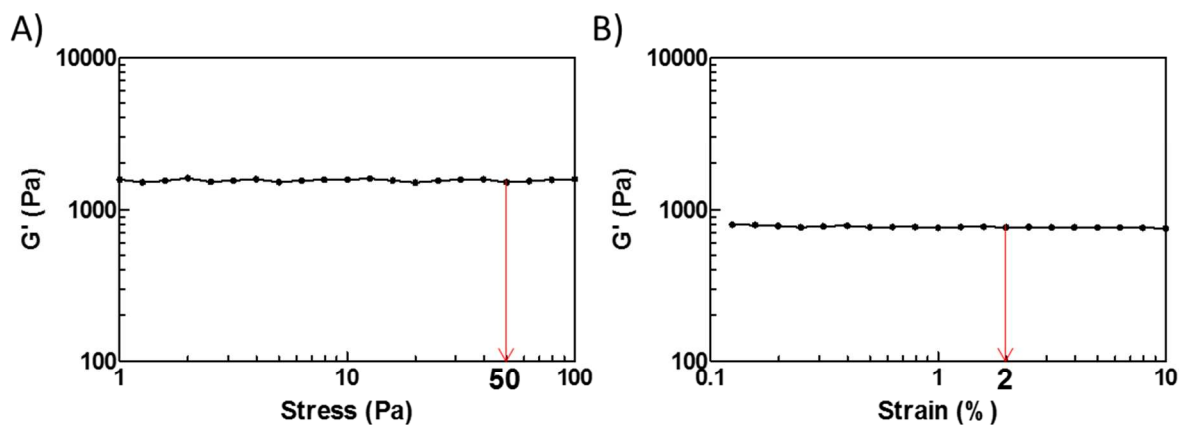


Figure S2. Results of A) a stress sweep from 1 to 100 Pa in oscillatory rheology at 1 rad/s on the photorheometry apparatus based on a stress-controlled rheometer (AR 1000) and B) a strain sweep from 0.1 to 10% on the strain-controlled rheometer that was used to measure the storage modulus of swollen hydrogels (ARES RFS). The results confirm that all of the oscillatory conditions used in this study are in the linear viscoelastic range: The red arrows show the stress amplitude used during photorheometry experiments and the strain amplitude used for subsequent analysis after cure.

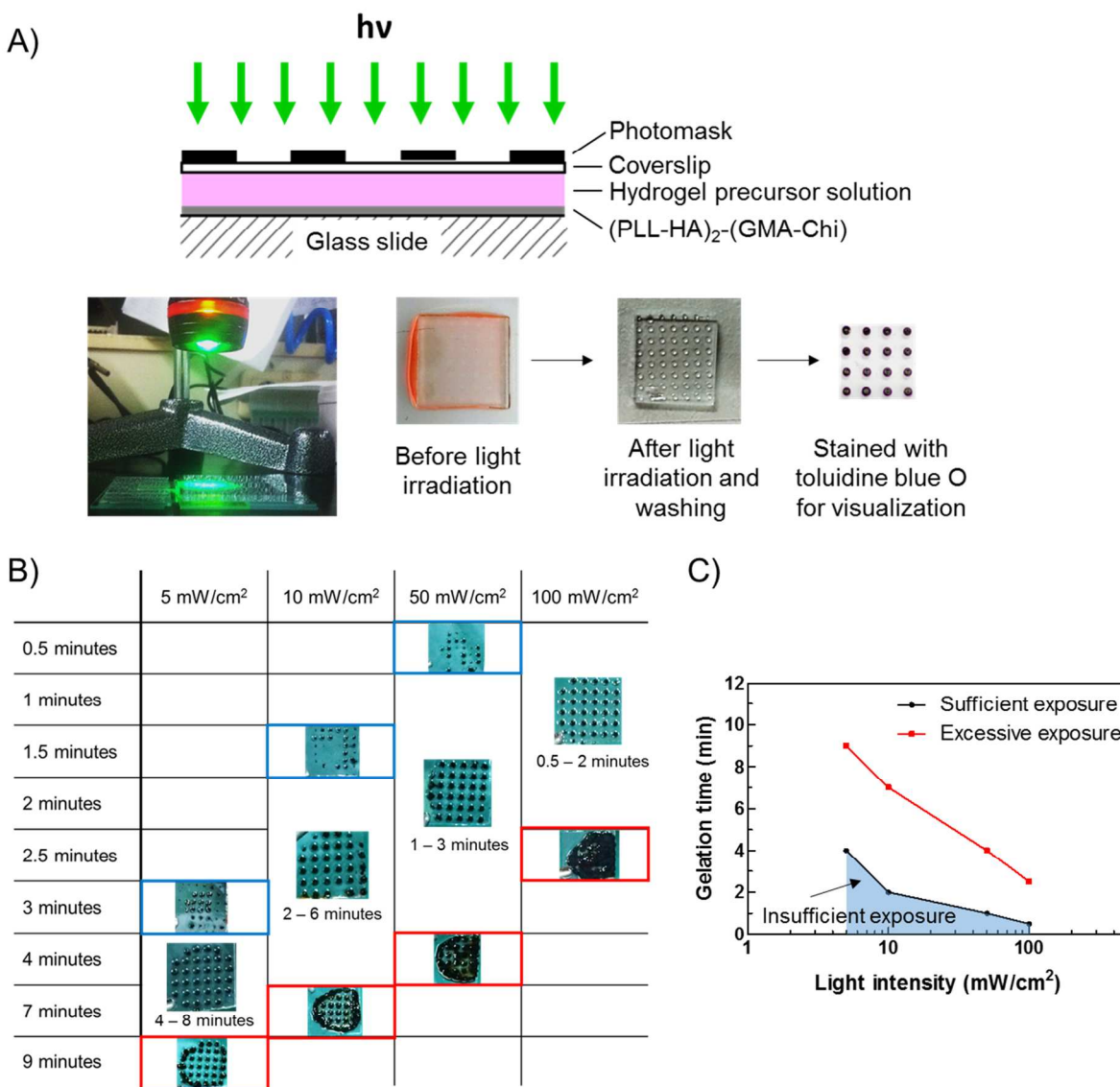


Figure S3. A) Schematic representation of the micropatterning process. The hydrogel precursor solution is applied onto the (PLL-HA)₂-(GMA-Chi)-modified glass slide, bounded by a 320micron thick Teflon spacer. A cover slip is placed over the gel and a photomask is place on top of it. Thus, irradiation is performed from the side in contact with an untreated glass surface. The LED source is placed over the sample and turned on for the desired irradiation time. Then the mask and coverslip are removed (in cases of insufficient exposure, small islands of gel are on the coverslip). The (PLL-HA)₂-(GMA-Chi)-modified glass slide is rinsed with PBS, and then

stained. B) Representative photographs of microgels formed using different light intensities and exposure times. Images indicative of insufficient exposure are outlined in blue; images indicating that gels had undergone “excessive exposure” are outlined in red. C) Graphical representation of the minimum exposure that produces a well defined pattern on the (PLL-HA)₂-(GMA-Chi)-modified glass slide (black curve), corresponding to “sufficient exposure” in the body of the manuscript and the upper bound on exposure time so that disks of gel are separate (red curve). [Heparin] = 3.33%, linear [PEG-DA] = 6.67%, [EY] = 0.01%, [TEOA] = 0.1%, in PBS (pH 7.8).

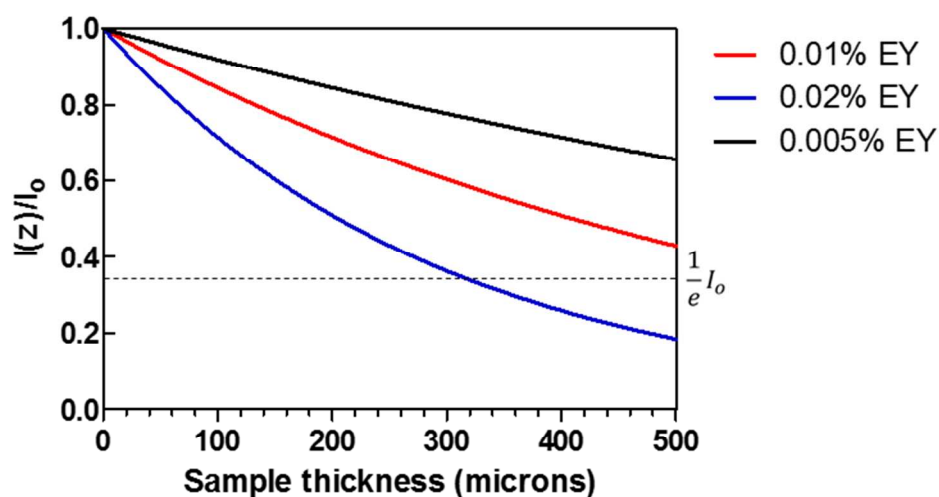


Figure S4. Intensity profiles calculated using the molar absorptivity of EY ($1.09 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$) for thickness up to that of the thickest samples used in this study (rheometry samples were 500 microns thick, while the micropatterning samples were 320 microns thick). The fraction of incident light that reaches the back of the sample depends on the concentration of the photo-initiator EY. The optical penetration depth, L_p , defined as the depth at which the initial light intensity is attenuated by $1/e$ (dotted line), which decreases from 1200 microns for 0.005% EY to 300 microns for 0.02% EY.

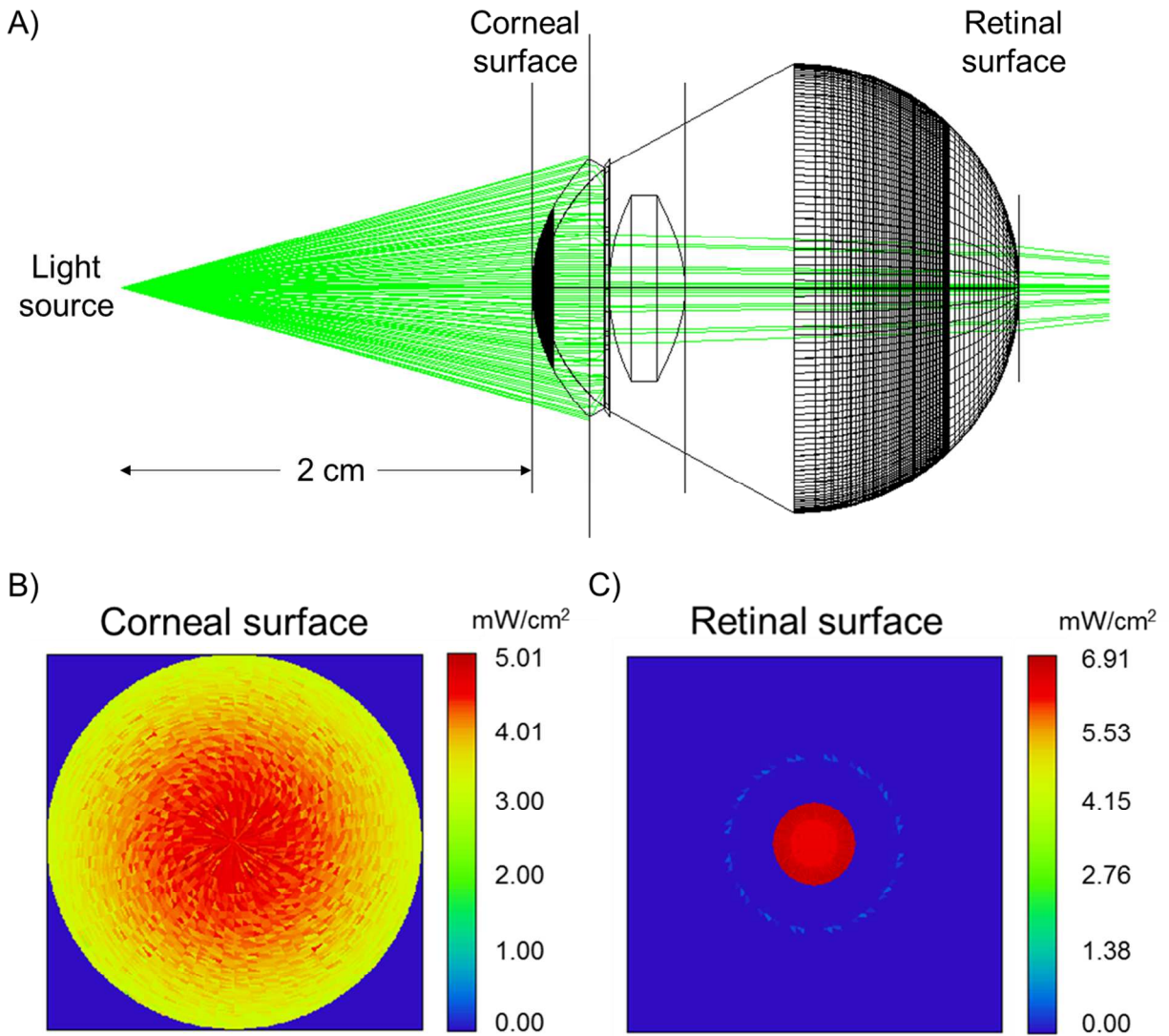


Figure S5. Computational eye model using ZEMAX ray-tracing software. (A) A single LED (32° viewing angle, 525 nm) was placed 2 cm away from the cornea on axis. The number of rays from the light source was set at 10 million. A corneal irradiance of 5 mW/cm^2 (B) corresponds to a retinal irradiance of 6.9 mW/cm^2 (C).

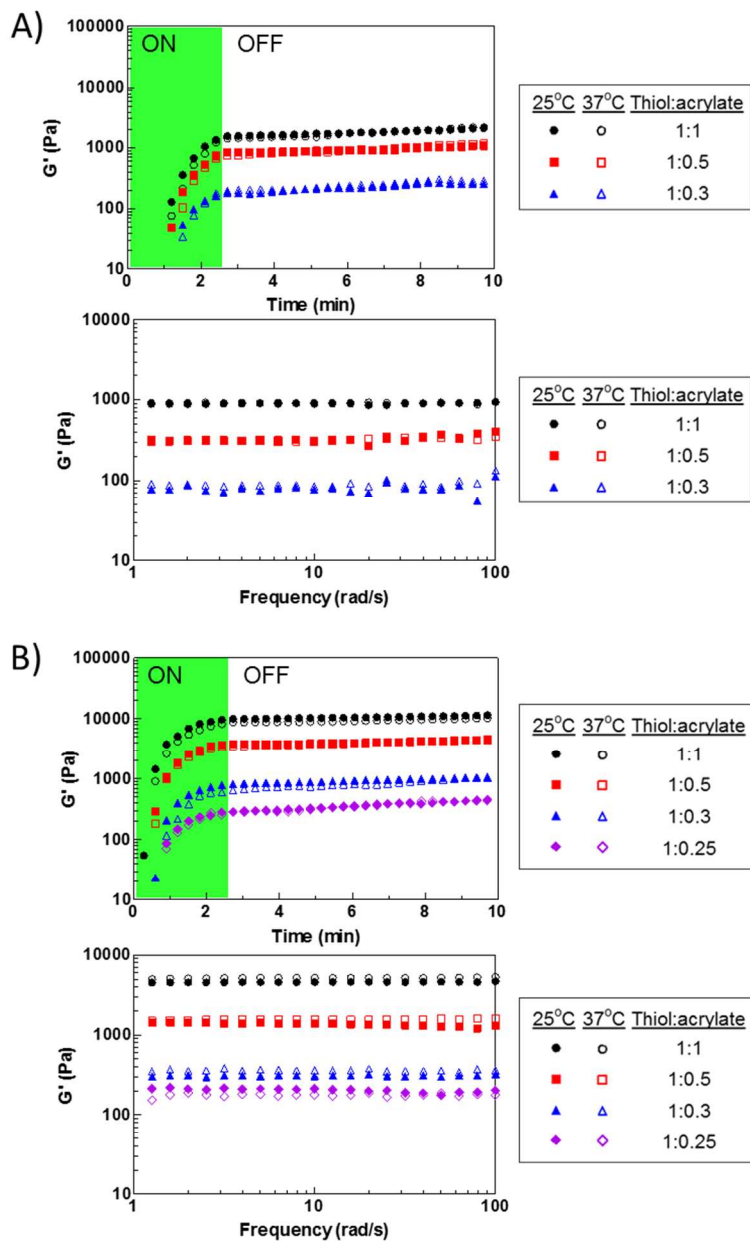


Figure S6. Gelation kinetics and final gel moduli of hydrogels prepared at different crosslinking conditions using linear PEG (A) and 4-arm PEG (B), other parameters held fixed in PBS (pH 7.8). There is no significant difference in gelation time or final swollen modulus between hydrogels prepared at 25°C and 37°C. Gel solution was irradiated with a 525nm green light at 5 mW/cm² for 2.5 minutes.

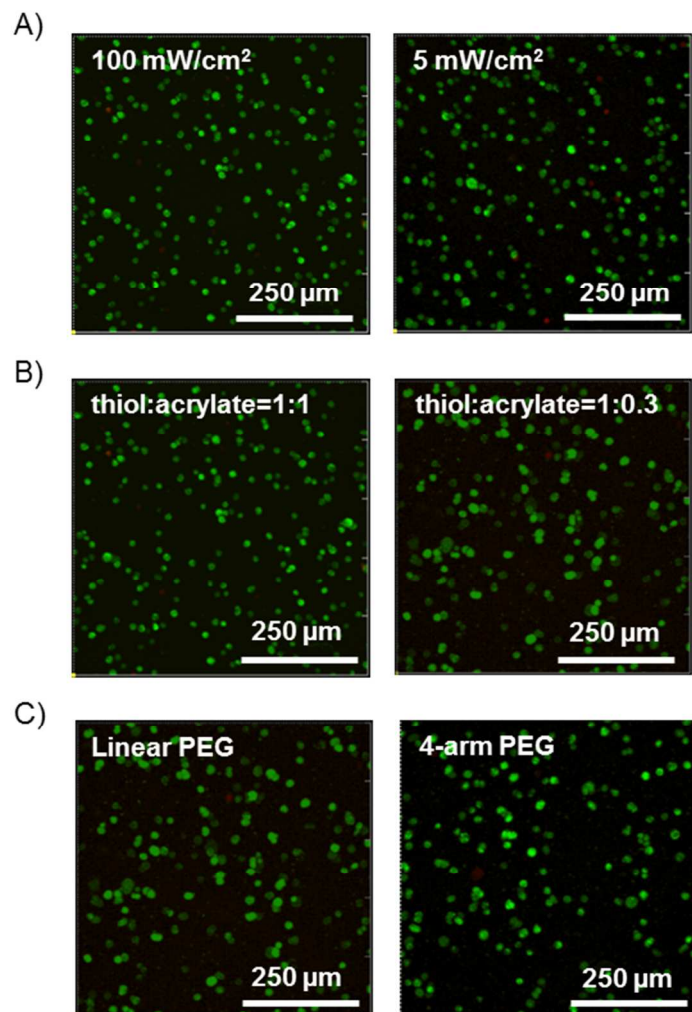


Figure S7. Cell viability of 3T3 fibroblasts (2×10^6 cells/mL) encapsulated inside heparin-based hydrogels at different (A) light intensities, (B) crosslinking densities and (C) PEG architecture. Live cells are stained green and dead cells are stained red. Cell viability in all cases was $\geq 96\%$. Unless otherwise noted, hydrogels were prepared with 2.33% heparin (w/v), thiol:acrylate 1:1, 0.01% EY and 0.1% TEOA in PBS (pH 7.8) with linear PEG and 5 mW/cm² irradiation.